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Genetic Targeting:
The Third Wave in Therapeutic Development

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Genetic targeting represents an inevitable, major step in the evolution of human therapeutics: the direct intervention of pharmaceuticals at the source-code of disease, the genetic sequence. This approach can potentially address any disease attributable to the functioning of a deleterious gene or genes. Several unique qualities make genetic targeting an important development in the history of human therapeutics: the potential for unparalleled specificity, systematic drug development, broad disease applicability, and proprietary compounds.

Introduction

The NIH has been the engine behind most of the advances in biotechnology and genetic targeting, through both intramural and extramural programs. This conference (see Acknowledgments) is a clear demonstration of the integral role that government can play in propelling new technologies both for human benefit and for industrial growth.

This is a tradition that dates back at least as far as the late 1930s, when the United Sates government played a pivotal role in developing penicillin, which had languished as a research oddity for well over a decade. I think we have learned a lot since the early days of penicillin.

The researchers gathered together for this conference have a great deal to be proud of, as pioneers of a difficult scientific arena that

holds enormous promise. Certainly the industrial enterprises represented here are in their debt for the scientific ground they have tilled.

Genetic Targeting

There are three topics that I would like to address. First, I would like to indicate why the field of sequence-selective gene inhibition, or genetic targeting, is such an enormous scientific and industrial opportunity, even in the face of clearly daunting technical hurdles. Second, I would like to describe how I believe the field will evolve in terms of actual products. And third, I would like to offer a few ideas as to where I think this field will be five years from now.

We use the term genetic targeting to describe our company's mission, rather than "antisense oligonucleotides," because the latter is but one of a number of mechanisms we seek to employ over time to accomplish sequence-selective gene inhibition. Genetic targeting means "therapeutics that target DNA or RNA with sequence specificity."

Our goal is to develop pharmaceuticals that intercept the activity of disease-causing genes not at the protein level, where most conventional pharmaceuticals act, but at the level of the DNA or RNA responsible for the early events in gene expression. These DNA and RNA targets are generally present in much lower copy number than their corresponding proteins, and the structure of these targets, as defined by their sequences, is well understood, relative to protein structure (Figure 1).

We view genetic targeting as a third wave in the development of human therapeutics. The first wave has been based primarily upon the empirical screening of compounds for biological activity. This approach, although it has formed the basis of most of the pharmaceutical industry, is limited by the inherent "hit-or-miss" nature of the screening process (Figure 2).

The second wave, that of the biotechnology industry, has employed the tools of recombinant DNA and cellular expression systems to manufacture proteins having therapeutic activity. These techniques

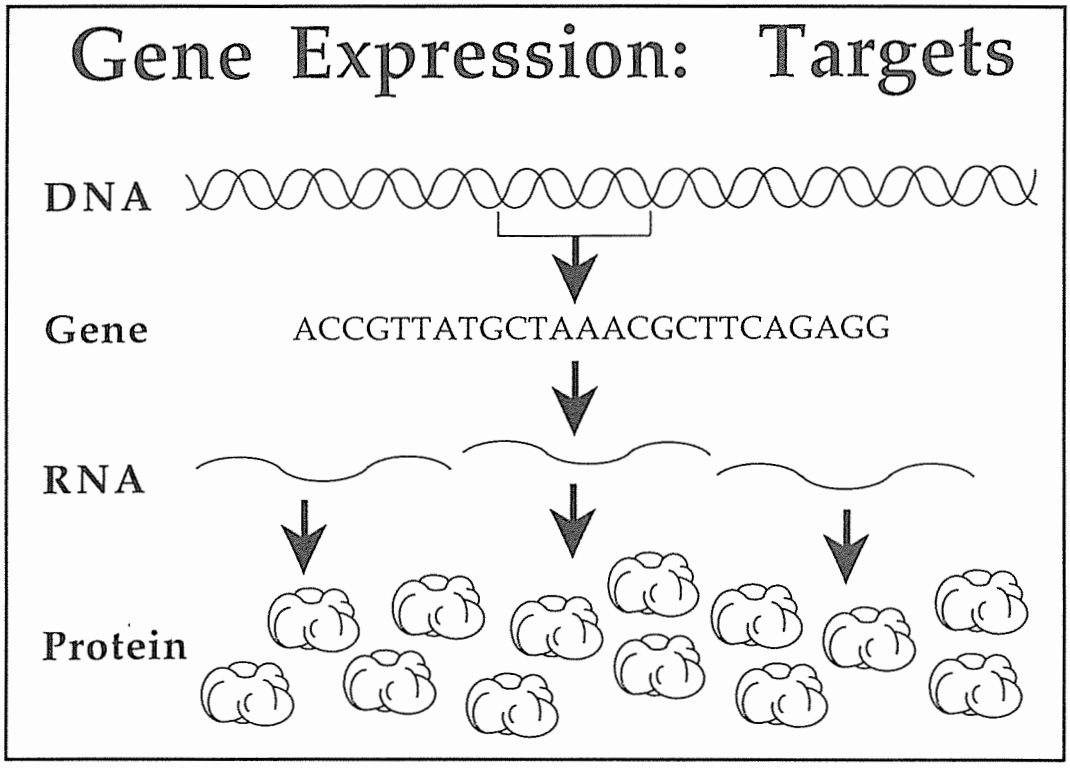

Figure 1. Gene Expression: Targets. ©Gilead Sciences, Inc.

make possible the production of a category of biological molecules that otherwise would be unavailable; however, they are inherently limited in that the roster of therapeutically feasible proteins is relatively short. As a result, there are well over 100 companies pursuing the development of approximately 30 therapeutic proteins.

The third wave, genetic targeting, employs recent advances in synthetic organic chemistry and molecular genetics to develop pharmaceutical agents, not proteins, directed against the genetic sequences underlying a broad array of diseases.

Having been in the venture capital business previously, I am accustomed to asking of any new, compelling idea, "If it is so appealing, why hasn't it happened already?"

The prospects for sequence-selective gene inhibition were hinted at several years ago; for example, with the pioneering PNAS articles from Paul Zamecnik's lab[1] in the late 1970s. However, several critical developments have only recently made genetic targeting possible on a practical basis. As indicated on the right-hand side of the pyramid (Figure 3), the sophisticated tools of molecular genetics have been

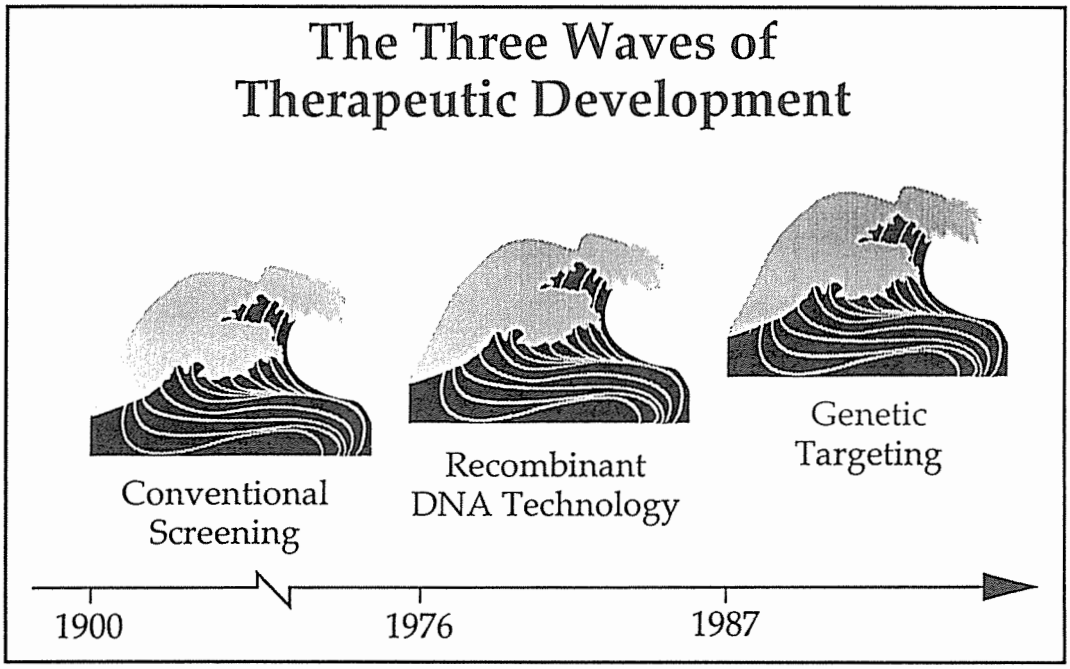

Figure 2. The Three Waves of Therapeutic Development. ©Gilead Sciences, Inc.

applied successfully to identify the roles of specific genes in viral diseases and certain forms of cancer. The sequences of many of these disease-causing genes have now been determined, providing the essential information for genetic targeting. The body of available sequence knowledge continues to expand rapidly.

As indicated on the left-hand side of the pyramid, the first generation of potential genetic targeting agents, oligonucleotides, has only been within the reach of synthetic organic chemistry since the early 1980s. The advanced techniques required to rapidly produce chemical variants of these agents, and to scale up the solid support synthesis techniques used to make oligonucleotides have opened up a relatively unexplored molecular size bracket in the human pharmacopoeia: the 3,000 to 10,000 molecular weight range, which lies above the typical 500 molecular weight cutoff for typical pharmaceuticals, and below the 20,000 to 200,000 range into which most protein therapeutics fall.

Despite these recent technical foundations, genetic targeting presents numerous tough technical hurdles, as our company has found in our research and development efforts. Why then pursue it as a

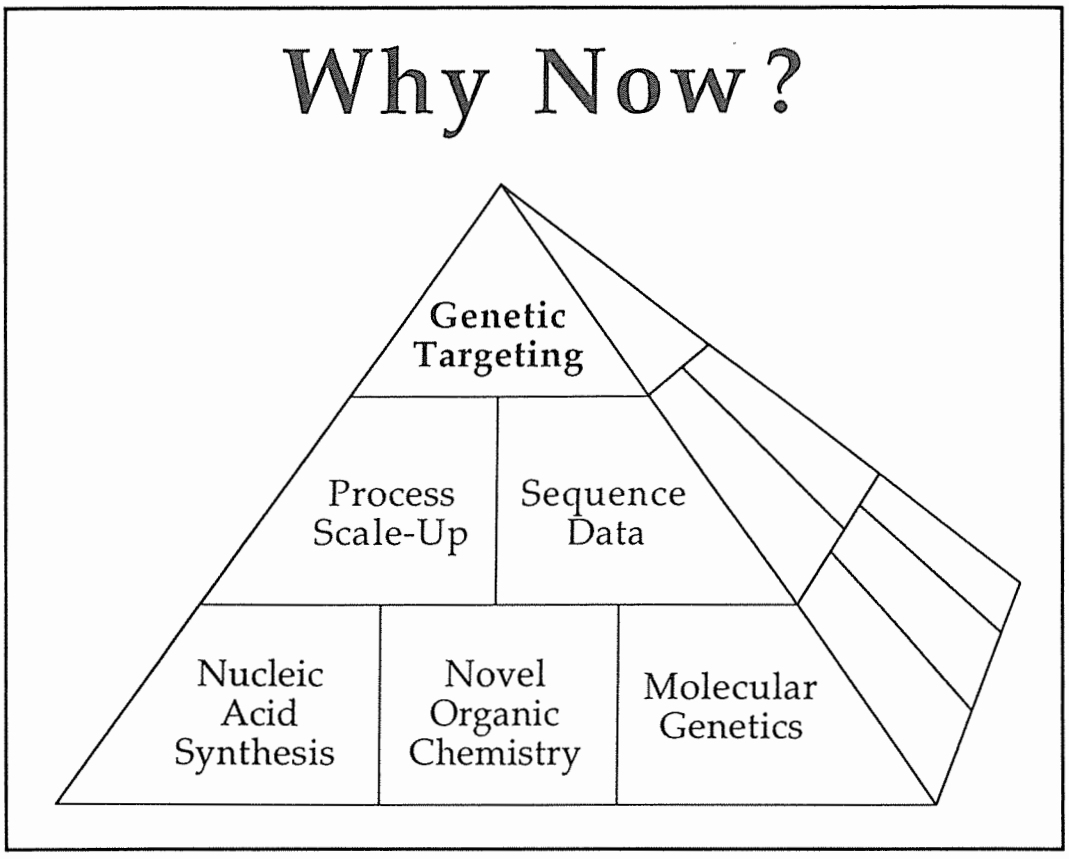

Figure 3. Why Now? ©Gilead Sciences, Inc.

commercial opportunity? The reason was succinctly summed up by Mr. Benno Schmidt, one of our directors, in his dedication address in May of this year for the new Rockefeller Laboratories at Memorial Sloan Kettering Cancer Center, of which Mr. Schmidt is Chairman. In his words, "the direct intervention of pharmaceuticals at the source-code of the disease, the genetic level, makes it at least theoretically possible to address any disease attributable to gene malfunction."

The list of such diseases is long, ranging from viral diseases and certain types of cancer to other pathogen-derived diseases, autoimmune disorders, neurodegenerative disorders, and chemotherapy resistance.

The potential advantages of genetic targeting for drug-development companies are rather easy to identify. The attractions of unique specificity, a target-rich environment of previously "untreatable" diseases, and of course patentability, have prompted several such companies to contact some of the groups working in this field

regarding possible partnerships. For the handful of young enterprises in this arena, collaborations with larger companies may be essential in order to meet the enormous costs of pharmaceutical development.

The ongoing challenge for most pharmaceutical companies is the successful development of follow-on products. Throughout the history of the pharmaceutical industry most companies have achieved success on the basis of a single drug. In fact, many such companies still depend heavily on the sales and earnings performance of only one or two drugs. For example, Syntex's Naprosyn™ (Syntex, Palo Alto, CA) products account for some 70 percent of its $1.2 billion in sales. Similarly, Glaxo's primary drug, Zantac™ (Glaxo, United Kingdom), represents one-third of its $3.3 billion in sales.

Therefore, perhaps the chief attraction for pharmaceutical companies of genetic targeting is the prospect of systematic drug development, whereby not just one drug, but a family of disease-specific drugs can be generated, providing a fuller pipeline of potential new products, and hence diminishing the revenue impact of blockbuster drugs coming off patent or falling prey to competing products.

By what means can genetic targeting potentially be accomplished? Clearly there are tantalizing biological data indicating that antisense oligonucleotide analogs can selectively inhibit gene expression by disrupting RNA processing or translation. The exquisite specificity of Watson-Crick base-pairing depicted in Figures 4 and 5, we now know can be employed, in some circumstances, to effectively disable a single-stranded target *in vitro*.

Clearly antisense oligonucleotides, which generally have RNA as their target and can be derivatized into countless analogs, including those to which are appended functional groups to enhance cell permeation or hybridization affinity, are the most accessible genetic targeting agents in the near-term. It may even be possible to confer upon an antisense oligonucleotide the capacity to cleave its target, using mechanisms analogous to those found in catalytic cleavage of RNA by ribozymes.

There are other prospective means of selectively inhibiting gene expression, several of which are on our company's research agenda.

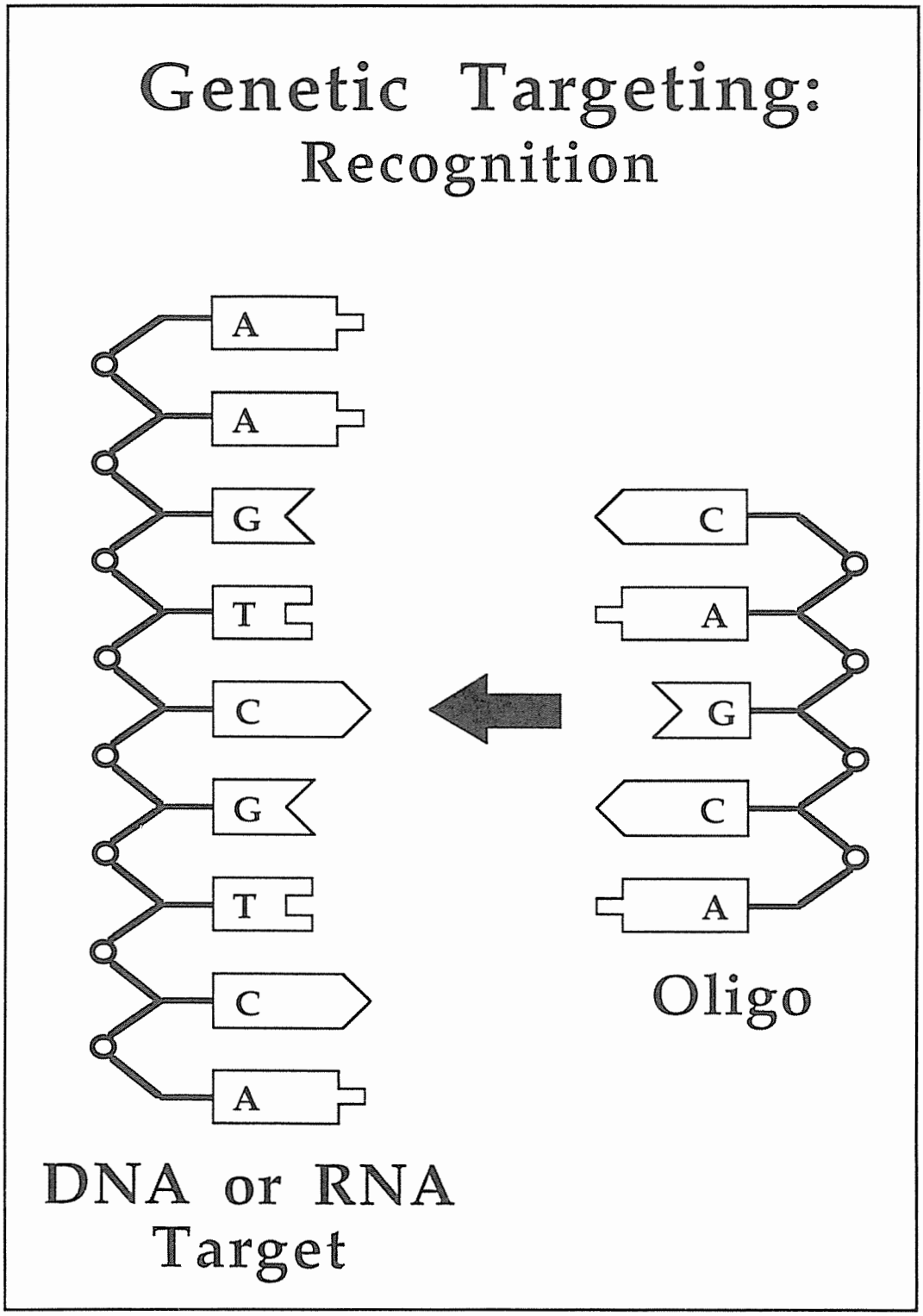

Figure 4. Genetic Targeting: Recognition. ©Gilead Sciences, Inc.

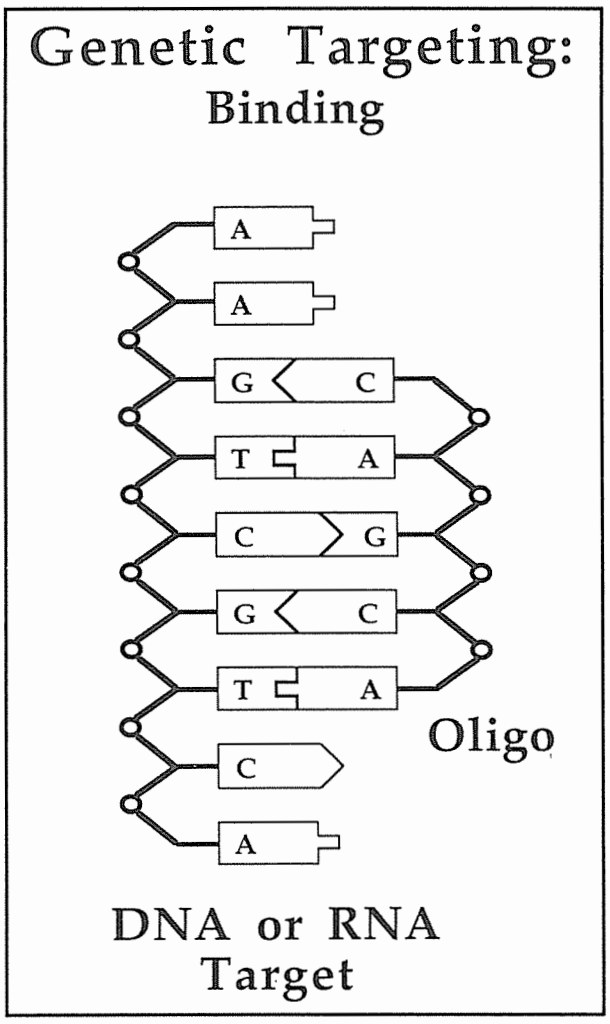

Figure 5. Genetic Targeting: Binding. ©Gilead Sciences, Inc.

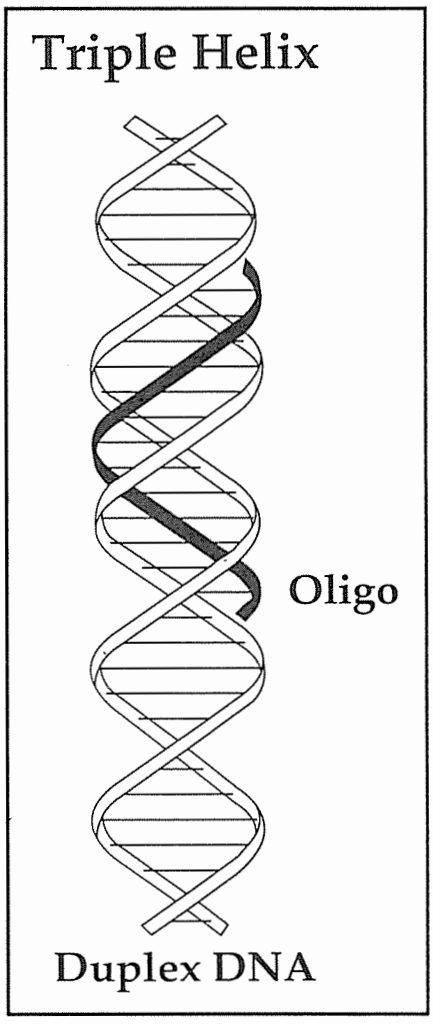

Figure 6. Triple Helix. ©Gilead Sciences, Inc.

They include triplex formation on double-stranded DNA, minor groove agents having enhanced sequence-specificity, vector-mediated antisense inhibition (which we regard as a longer-term prospect, given the added biological and regulatory complexity it entails), and, finally, a category never to be overlooked: *whatever else works.* Fortunately these different approaches are very complementary in terms of the chemistry, molecular genetics, and cellular biology techniques they require, and thus can be jointly addressed by a relatively small, focused research group having the necessary types of expertise.

As we have come to realize, triple helix formation is incompletely characterized as a potential means of selective gene inhibition,

but it does offer potential advantages (Figure 6). For example, there are fewer copies of a DNA target than of its corresponding RNA transcripts, suggesting that lower doses of a sequence-targeting agent may be feasible. Another potential advantage is the ability to directly address integrated DNA of latent viruses.

Is this pie-in-the-sky stuff? Is it simply asking too much for an intravenously administered molecule the size of a 15-base oligonucleotide (5,000 molecular weight) to enter virtually all cells, proceed to the nucleus, enter that, find its way through chromatin, and then bind to a tangled stretch of chromosomal DNA? That is asking a lot.

There are, however, drugs in use today which do exactly what I have described, and which are relatively large molecules. An example is bleomycin, an intravenously administered anti-cancer agent having a molecular weight greater than 1,400. Bleomycin permeates most cells, penetrates the nucleus, binds to DNA, and then causes DNA strand scission. Like all anti-tumor agents, it is relatively indiscriminate as to cell type and has numerous associated toxicities. Wouldn't it be advantageous to have a molecule that does what bleomycin does, but is selective as to what chromosomal sequence, and hence cell function, is affected?

The question often arises whether chromosomal DNA would be accessible to a 5,000 molecular weight molecule, given the hindrances of chromatin or other DNA-associated proteins. I think the answer lies in simply recalling that every transcriptionally active gene is rapidly being scanned by a comparatively massive RNA polymerase complex having an effective molecular weight in the range of 500,000, like a large locomotive racing down a train track. For transcriptionally active genes, the issue is not accessibility, but the relative binding affinity of the therapeutic agent for its appropriate target.

What about the costs of synthesizing and purifying oligonucleotides, whether they are intended for antisense inhibition or for triplex binding? The same questions of cost were prominent in the early development of penicillin. At the time of the first test of penicillin's efficacy in a human, the material, which by the way was highly impure, was so precious and so difficult to obtain in volume that it was

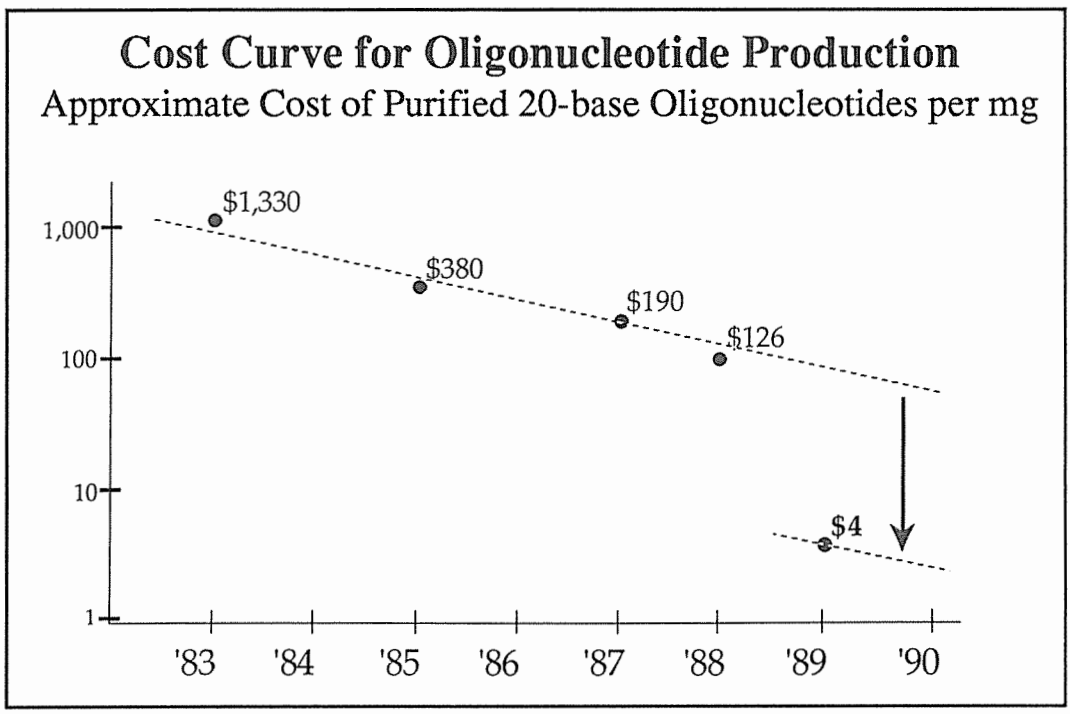

Figure 7. Cost Curve for Oligonucleotide Production. ©Gilead Sciences, Inc.

recovered from the patient's urine for reinfusion. Treatment of that first septic patient in fact had to be discontinued for lack of compound, and the patient, who had been responding to the therapy, died. I am sure we are all grateful that the development of penicillin was not abandoned because of cost considerations.

Historically, oligonucleotides have been synthesized in minute quantities for molecular biology research purposes on automated DNA synthesizers. Thus, going back to 1983, microgram amounts were made. In the absence of any substantial impetus to increase the scale of synthesis (since the molecular biologists only need sub-micromole quantities which they radiolabel for easy detection), we saw only modest increases in the amount of material that was routinely made.

We estimated the historical costs per milligram of a purified 20-base oligonucleotide based upon product literature from vendors of custom oligonucleotides up to 1988, as depicted in this graph (Figure 7). Necessarily, these are very rough approximations. The production cost curve from 1983 through 1988 has a profile similar to that of most

manufacturing processes: production costs decline in a log-linear manner over time as the basic production process undergoes incremental improvements, and as experience is accumulated. Remember that during this period there was no impetus for making large amounts of oligonucleotides.

Between 1988 and 1989 there is a large discontinuity, reflecting deliberate attempts by our group to substantially increase the scale of synthesis, using new chemistries, and benefiting from economies of scale. Again, the latest data point, for 1989, is a rough approximation. I am sure that a few other groups who have attempted scale-up have seen a similar jump to a lower cost curve. I will be surprised if we do not in the near future see further lowering of the curve.

Ideally, however, cost will be made a subordinate issue because of marked reductions in dose requirements, as more effective analogs are developed. The extracellular concentrations at which effective gene inhibition is observed *in vitro* today imply non-feasible dosing requirements for all but the most severe and intractable diseases, such as AIDS.

How difficult is it to improve parameters that would lead to a lower effective dose, parameters, such as binding affinity, stability *in vivo*, and cell membrane permeability? For the most part, we find it is very difficult. For example, our group has made and tested numerous analogs which by various design criteria should have increased binding affinity to a single-stranded target, but did not. It has also been much harder than anticipated to construct reliable, rigorous cellular assays for detecting selective gene inhibition across several different tissue types.

Fortunately there are some bright spots as well. It now appears that there are several types of conservative chemical modifications that can effectively prevent degradation of oligonucleotides in serum and in the cytosol. It also appears that other types of chemical derivatives we now make enjoy markedly enhanced cellular permeation, and there is rapid progress on the scale-up front.

On balance, the road is difficult, but if it weren't, the world wouldn't need the dedicated groups that will undoubtedly make substantial progress in this field.

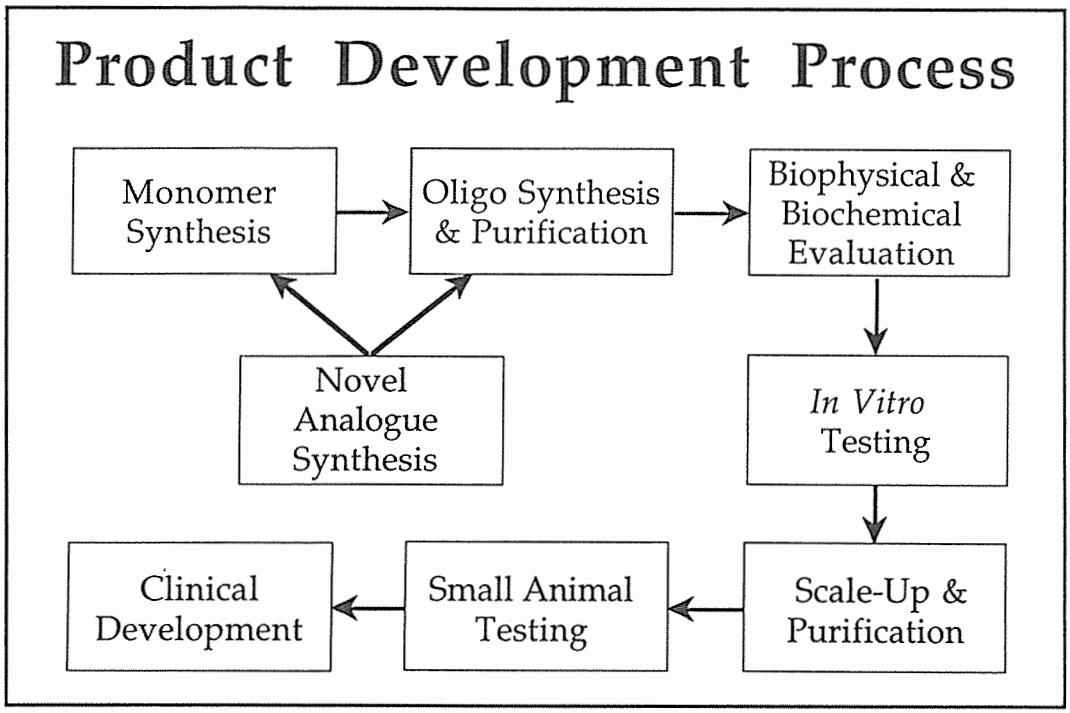

Figure 8. Product Development Process. ©Gilead Sciences, Inc.

I believe that the most rapid progress can be achieved through an integrated inhouse system of synthesis and evaluation that resembles in some respects a production line, and which requires relatively rare skills in synthetic organic chemistry and molecular biology (Figure 8). The scheme is not unlike that exploited by Genentech in the early days of biotechnology. In a production-line manner, that company was able to rapidly identify a promising protein, isolate its gene, clone it, express the protein, evaluate it and then scale up its expression if it proved interesting. The competitive advantage in our case resides in the skill of the various teams, monomer production, oligonucleotide synthesis, molecular biology, and the rapidity with which they can reach yes-no decisions on each new chemical entity.

Where will the field of genetic targeting be in five years? Within that time frame I think molecular biologists will enjoy the use of a new array of reliable reagents with which to selectively inhibit gene expression and hence dissect the function of innumerable genes. Clearly these reagents are not in hand now.

In five years we will have descended two more logs on the production cost curve for oligonucleotides.

Novel triplex-forming oligonucleotides, not restricted as to the sequence composition of the target, will have been developed. That is, the code for triplex recognition will be successfully extended.

Agents targeted against specific sequences of DNA will be shown capable of blocking the binding of tissue-specific transcription factors, opening a new pathway for therapeutic gene modulation.

The multigenic events underlying several major forms of cancer will be characterized with precision. Genetic targeting agents directed against one or two implicated sequences in these cancers will be shown to inhibit malignant growth with a specificity unheard of among present-day cancer therapeutics.

Other diseases, such as the autoimmune variants of rheumatoid arthritis and diabetes, will be traced to aberrant expression of a small number of discrete genes, and thus potentially be addressable via genetic targeting.

In the process of exploiting genetic targeting, we will have expanded, in a major way, our understanding of bioorganic chemistry, cell membrane events, and the mechanisms of gene expression itself. I think we will also, in the process, build several major enterprises.

In a recent speech in Montreal, the Director of the National Cancer Institute, Dr. Samuel Broder, pointed out that several of the advanced approaches now being applied to HIV therapy, including antisense oligonucleotides, only two years ago *"would have been labeled science fiction."* If that sort of progress can happen in two years, I think we will all have extraordinary progress to report when we convene five years from now.

Acknowledgments

This paper was originally presented as a speech at the National Institutes of Health Antisense Oligonucleotides Conference on June 20, 1989.

I would like to thank the organizers of the NIH Antisense Oligonucleotides Conference, as well as their colleagues at the NCI and NIAID, for taking such a proactive role not only in sponsoring this

meeting but in playing a larger catalytic role in fostering scientific advances in the field.

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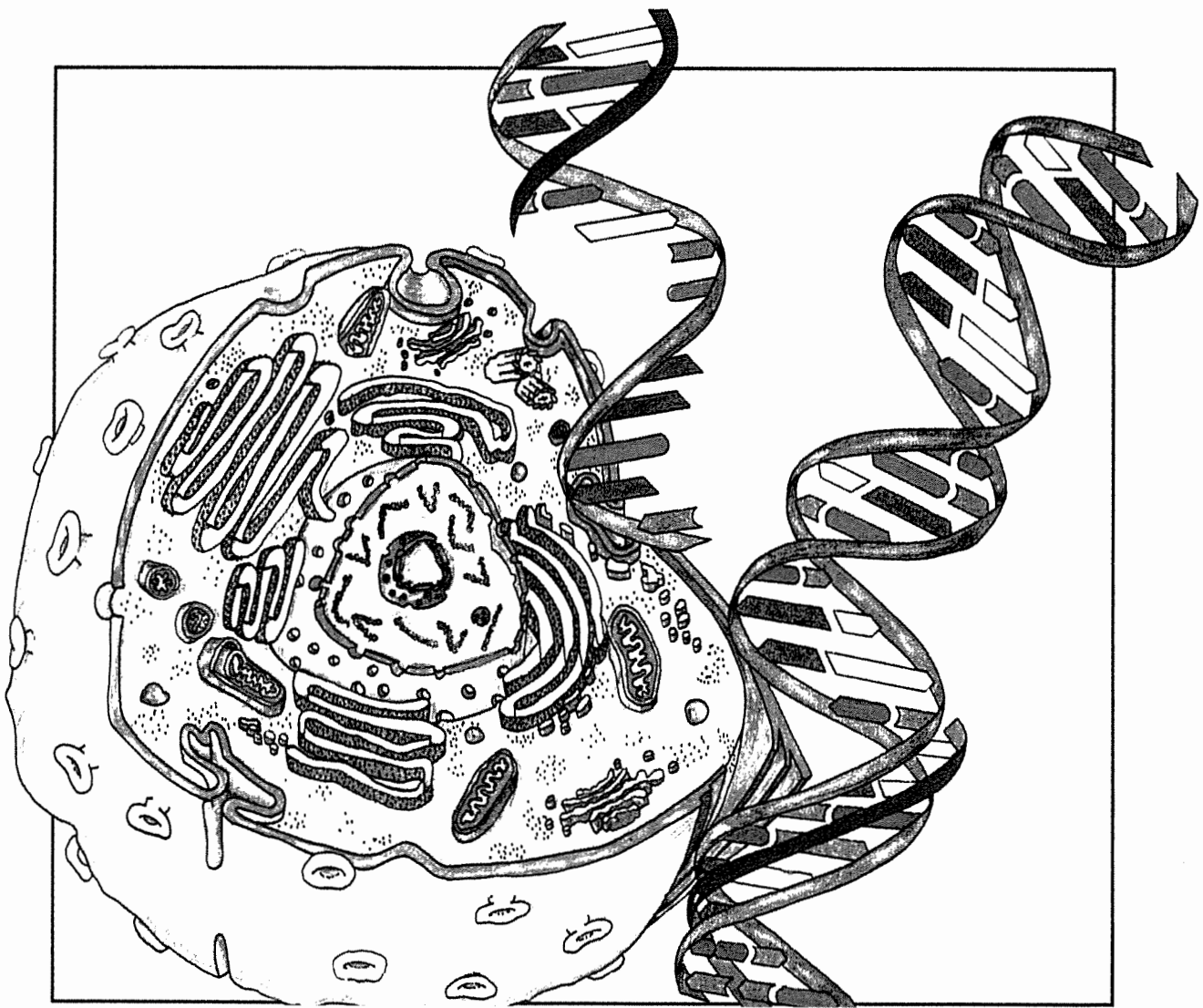
Volume 2

DISCOVERIES IN ANTISENSE NUCLEIC ACIDS

EDITOR
Christine L. Brakel



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APPENDIX

Companies With Antisense Programs and/or Gene Delivery Technology

Antisense Technology

Gilead Sciences, Inc.
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Genta, Inc.
Microprobe Corp.
Triplex Pharmaceutical Corp.
Antivirals, Inc.
Sense Technologies, Inc.

Oligonucleotide Synthesis Companies

Synthetic Genetics, Inc.
Genetic Designs, Inc.
SyntheCell
Glen Research Corp.

Transgenic Technology

Genetic Therapy, Inc.
Somatix
Viagene, Inc.

Other Companies

Alleix
Applied Biosystems Inc.
Cetus
Chemex
Du Pont
Enzo Biochem, Inc.
Glaxo
Monsanto
Sterling Drug/Kodak

Antisense Technology

Gilead Sciences Inc. *(Foster City, CA)*

Gilead was the first company funded by major venture money to be dedicated to the development of antisense products. Gilead was founded in August 1987 by Michael Riordan, M.D., MBA, around the technology being developed by three key scientists in the antisense field. These scientists, now on the company's scientific advisory board, are Peter Dervan from California Institute of Technology (expert in interactions between proteins and nucleic acids, and creation of DNA-cleaving and -binding agents), Douglas Melton from Harvard, and Harold Weintraub from the Fred Hutchinson Cancer Research Center in Seattle (the latter two were the first to show the feasibility of antisense inhibition in eukaryotic cells).

Short-term projects for Gilead include antisense oligomers directed at inhibiting the translation of viral and oncogene mRNA. Medium-term projects include antisense products, with the ability to cleave nucleic acids, directed against duplex DNA. Long-term projects include vector-mediated antisense gene insertion into cells for sustained production of antisense molecules.

The company was originally funded with $2 million from Menlo Ventures. A recent round of financing raised an additional $10 million. Participants were J.H. Whitney; Venrock; Willaim Blair; Merrill, Pickard, Anderson and Eyre; Vista Group; Abingworth Ventures; Concord Partners; and Hancock. The board is composed of Michael Riordan (president and CEO), DuBose Montgomery, and Donald Rumsfeld. There are approximately 20 employees in an 8,000-square foot facility. Michael Bigham, most recently from Hambrecht and Quist's Medical Ventures group, is CFO.

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